

A NEW TAXONOMY FOR *TRIFOLIUM VARIEGATUM* AND ITS RELATIVES

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ABSTRACT

Two related species of *Trifolium* L. endemic to western North America, *T. variegatum* Nutt. s. l. and *T. polyodon* Greene, are studied. Multivariate analyses and morphological differences suggest that three species should be recognized instead of two. *Trifolium variegatum* is divided into two species: *T. appendiculatum* Lojac. and *T. variegatum*. *Trifolium appendiculatum* (including *T. rostratum* Greene) is a large-flowered species endemic to California and southern Oregon. *Trifolium variegatum* is found throughout western North America and remains the most diverse, but is now circumscribed by a consistent floral morphology. *Trifolium polyodon* is restricted to the Monterey peninsula and has distinctive calyx lobe teeth; its circumscription remains unchanged.

Key Words: Fabaceae, section *Involucrarium*, *Trifolium appendiculatum*, *Trifolium polyodon*, *Trifolium variegatum*.

The genus *Trifolium* L. (the clovers, Fabaceae) are herbaceous plants with often trifoliolate leaves, papilionaceous flowers contained in racemose-umbellate heads to head-like inflorescences, diadelphous stamens, and persistent corollas. This genus has approximately 240 species worldwide (Zohary and Heller 1984). Its centers of diversity are the Mediterranean region and the parts of North and South America with Mediterranean-type climates. Several species, such as *Trifolium repens* L. and *T. pratense* L., are cultivated as forage crops (Zohary and Heller 1984), and thereby have become introduced and naturalized worldwide. A phylogenetic study of the genus based on nuclear ribosomal DNA ITS and chloroplast *trnL* intron sequences by Ellison et al. (2006) confirmed its monophyly.

Zohary and Heller (1984) recognized two lineages of New World clovers: species with a head subtended by an involucre of fused bracts, of which there are approximately 25, were placed in sect. *Involucrarium* Hooker; the remainder of New World clovers, those lacking the involucre, were placed in sect. *Lotoidea* Crantz. Ellison et al. (2006), using molecular data, placed all New World clovers into a more broadly circumscribed section *Involucrarium*. Interestingly, *Involucrarium sensu* Zohary and Heller, with the exception of one species, is contained in two clades emerging from a species-rich polytomy (Ellison et al. 2006). Regardless of whether the involucre is phylogenetically useful or not, it remains a useful field trait. Within *Involucrarium s.s.*, taxonomic importance has been placed on the appearance of the calyx, the involucre bracts, and stipules. Gross aspects of the morphology, such as leaflet shape, can often be misleading. The group consists of both annuals and perennials. Species can be widespread or local endemics. A small subset of these involucre clovers is separated from their counterparts by having narrow

calyx lobes uniformly longer than the calyx tube, a glabrous epidermis, and wheel-like involucre bracts. This includes the commonly-recognized species *T. wormskioldii* Lehm., *T. buckwestiorum* Isely, *T. polyodon* Greene, and *T. variegatum* Nutt. (Vincent and Isely 2012). These species form a clade in the Ellison et al. (2006) phylogeny. *Trifolium wormskioldii* is a perennial species, whereas the remainder of the species are annuals. *Trifolium buckwestiorum* is a rare species endemic to California and is distinguished by its cleistogamous flowers. Likewise, *T. polyodon* is only found in Monterey, CA and is separated from its close relative, *T. variegatum s.l.*, by having 8-14 calyx lobe teeth per calyx. *Trifolium variegatum* typically lacks calyx lobe teeth, but when they are present, have 1–8 calyx lobe teeth per calyx. It has, however, on occasion been lumped with *T. variegatum* (Isely 1993) and with *T. tridentatum* Lindl. (Jepson 1936). *Trifolium variegatum s.l.* is found throughout western North America, ranging from British Columbia in Canada south through the western U.S. (Arizona, California, Idaho, Montana, Nevada, Oregon, Utah, Washington, and Wyoming) and into Baja California, Mexico. It can grow from sea level up to 3,000 m in elevation, in habitats as diverse as vernal pools, chaparral, woodlands, and grasslands, as long as the area is locally moist. *Trifolium variegatum s.l.* and *T. polyodon* form the subject of the present study.

The taxonomy of *T. variegatum s.l.* and *T. polyodon* has been problematic. The group is circumscribed by an annual life cycle, glabrous epidermis, wheel-like involucre bracts, and uniformly-long calyx lobes. There is considerable phenotypic diversity. Various morphologies have been identified as worthy of specific recognition, with a resulting 16 epithets being proposed over the past 125 years. Proposed taxa were based on traits such as the number of flowers per inflorescence, the size of the vegetative structures, and

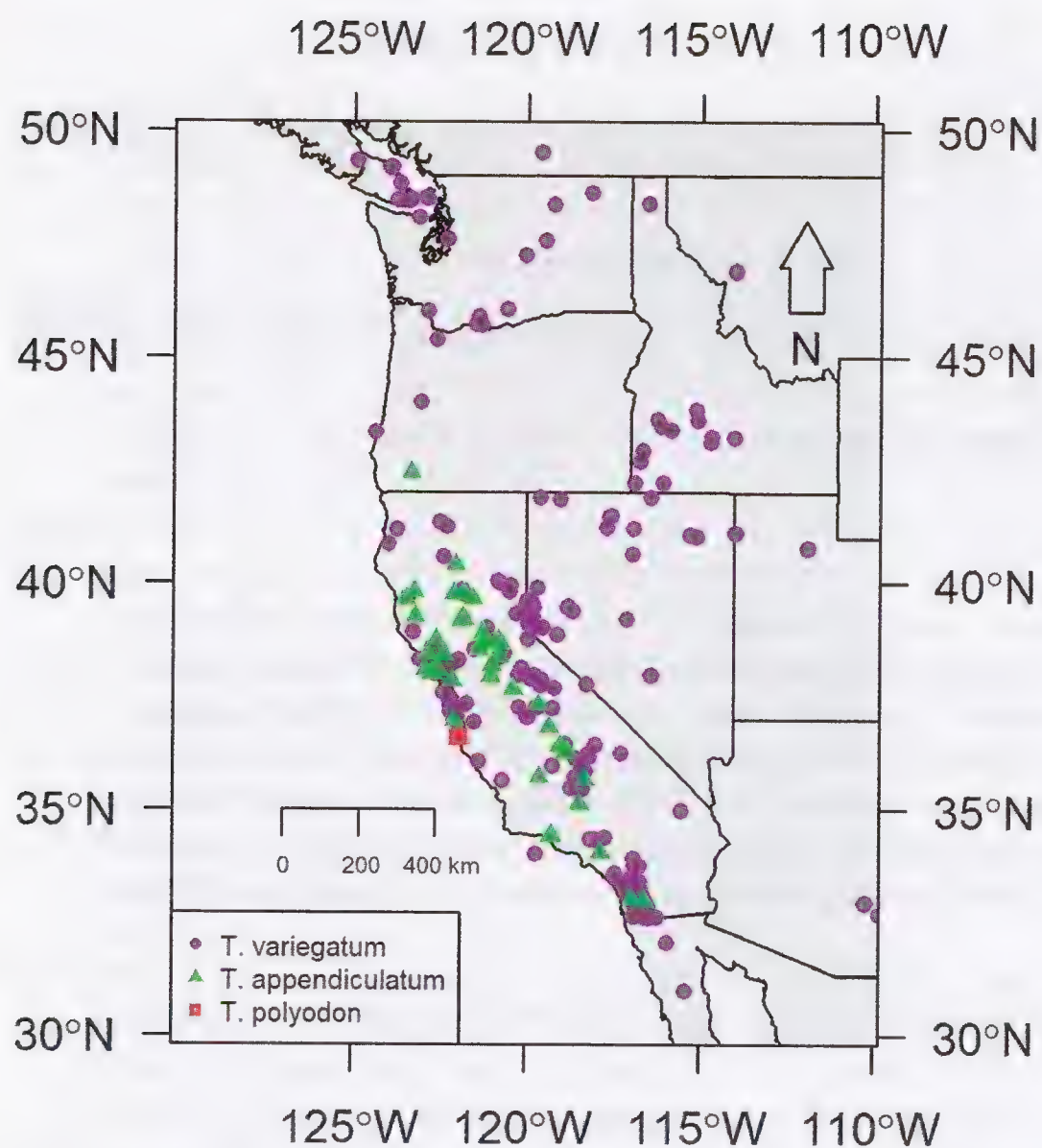


FIG. 1. Map of *T. variegatum* complex. Collections are mapped according to information derived from label data. Specimens are labeled according to taxa recognized in this study. Mapped specimens include those used in statistical analyses in addition to those not included.

peculiar floral morphologies. Recent publications covering this group include Zohary and Heller's (1984) monograph and the treatment of Vincent and Isely (2012) for Californian taxa. In both of these publications, *T. polyodon* is recognized as a distinct species. Each treatment divides *T. variegatum* into varieties. Zohary and Heller (1984) recognized two: *T. variegatum* var. *variegatum* and *T. variegatum* var. *rostratum* (Greene) J. Martin emend. D. Heller (nom. inval.; art. 30.8). These varieties are distinguished according to the absence or presence of a small beak on the keel petals of the corolla. Vincent and Isely (2012) recognized three varieties of *T. variegatum*: *T. variegatum* var. *geminiflorum* (Greene) Vincent, *T. variegatum* var. *major* Lojac., and *T. variegatum* Nutt. var. *variegatum*. The first variety represents a slighter and fewer-flowered form, the second variety has a more robust growth form, and the third variety shows the typical phenotype. Earlier treatments, such as the *Manual of Flowering Plants of California* (Jepson 1925) and *A California Flora* (Munz and Keck 1959), offer similar taxonomies, with the addition of a segregate: *T. appendiculatum* Lojac. This species roughly correlates to the previously mentioned *T. variegatum* var. *rostratum* (Zohary and Heller 1984) and *T. variegatum* var. *major* (Vincent and Isely 2012).

In order to clarify the taxonomy of this group, we assembled a number of herbarium specimens and investigated the breadth of its morphological and geographical diversity. This investigation of *T. variegatum* and its allies began with five groups (Foster 2015); their status as species was tested. After a multivariate analysis, three groups meriting recog-

nition at the species level were evident: *T. variegatum*, *T. appendiculatum*, and *T. polyodon*. In addition to traits described above, we identify one previously known and one novel character to aid in distinguishing these species.

METHODS

Specimens representing the entire range of this group (Fig. 1) were utilized for this study from the following herbaria: ASU, BM, BRY, CAS, ISC, MU, NY, MO, PH, RENO, SD, SRP, and WIS (for herbarium acronyms, see Thiers 2015). Dried specimens were examined under a dissecting microscope. Macroscopic linear measurements and angle measurements were made using a metric ruler and protractor, respectively. Microscopic characters were measured using a Wild MC3 dissecting microscope (Wild Heerbrugg, Heerbrugg, Switzerland) and a calibrated ocular micrometer. Flowers were rehydrated using 1% Aerosol OT (Fisher Scientific, Pittsburgh, PA; Ayensu 1967). They were then dissected in order to yield measurements for corolla, calyx, stamen, and pistil characters. A total of 275 specimens yielded measurements for all characters to be used in statistical analyses (see below). The following 27 characters were used in the analyses: stem diameter, stipule lobe length, petiole length, petiole diameter, leaflet base angle, terminal leaflet length, terminal leaflet width, petiolule length, petiolule diameter, head length, flower number, peduncle length, peduncle diameter, involucre lobe length, calyx circumference, calyx lobe length, calyx lobe width, calyx tube length, calyx lobe tooth number, banner petal length, banner petal width, wing petal lamina length, wing petal lamina width, keel petal lamina length, keel petal lamina width, connate filaments length, and style length.

Principal component analysis (PCA, Pearson 1901, Hotelling 1933) was carried out using R (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). PCA was performed using the *princomp* function, and projected in R. Characters selected for use in this analysis (all those listed above) represent a subset of all characters measured. They were selected for their consistency (it was easy to measure the same object at the same developmental stage for all specimens), variability (for some characters measurements were more or less invariant; these were excluded from such analyses [Sneath and Sokal 1973]), and their assumed developmental independence (logically correlated characters were left out, those highly correlated but of different organs remained in the analysis [Sneath and Sokal 1973]). Maps were produced in R (R Core Team 2013) using the packages *maps* and *mapdata*.

RESULTS AND DISCUSSION

The multivariate analysis (Fig. 2) indicates the presence of two groups. In the projection of principle

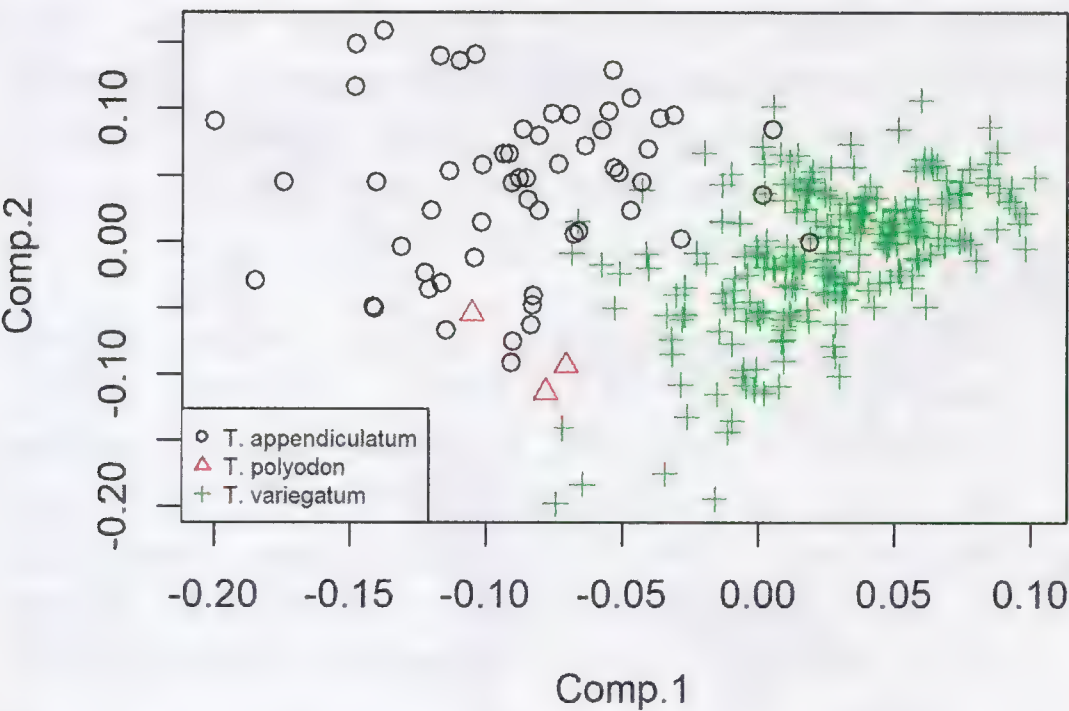


FIG. 2. Principal components analysis projection of all *T. variegatum* complex. Operational taxonomic units (OTU's) are labeled according to recognized species. Principle Component 1 (Comp. 1) explains 50.9% of variation, Principle Component 2 (Comp. 2) 11.2%.

component (PC) 1 and PC 2, the operational taxonomic units (OTU's) of *T. polyodon* are intermixed with those of *T. appendiculatum*. The characteristic morphological feature of *T. polyodon*, calyx lobe teeth, is included in the PCA, but has its highest loading on PC 7. The majority of OTU's have a character state of "0" for the number of calyx lobe teeth, so this character is ranked by the analysis as unimportant when considering morphological variation as a whole. A closer look at this character reveals that *T. polyodon* exceeds its relatives in terms of the number of calyx lobe teeth (*T. polyodon*: 8-14 teeth/calyx; *T. variegatum*: 1-8 teeth/calyx; *T. appendiculatum*: no teeth) and the average length of these teeth (*T. polyodon*: 0.1-3.1mm; *T. variegatum*: 0.1-1.4 mm; *T. appendiculatum*: no teeth). Thus, *T. polyodon* remains a separate species.

Disregarding *T. polyodon*, the two groups indicated in the multivariate analysis remain. The character loadings indicate a difference in general plant size. In PC 1, along which the groups are divided, every character has a loading of at least 0.1, and all loadings are oriented in the same direction. Thus, plants on the larger end of the spectrum are grouped into *T. appendiculatum*, while those smaller are called *T. variegatum*. The floral characters considered on their own depict a clearer separation, with *T. appendiculatum* having larger floral features, especially those of the corolla, than *T. variegatum*. In comparison, the banner petals of *T. appendiculatum* range from 6.3 to 15.8 mm in length, while those of *T. variegatum* range from 3.2 to 9.7 mm.

There are several other characters, not used in the multivariate analysis, that segregate *T. appendiculatum* from *T. variegatum*. A number (approximately 30%) of the specimens of *T. appendiculatum* presented a unique floral trait. The keel petals of these specimens have an appendage (hereafter referred to as a "beak") at their apices extending a few tenths of a millimeter. This beak, in fact, is the reason Lojacono gave *T. appendiculatum* its name (1883), and has been noticed by many botanists (e.g., Greene 1895; Heller 1905).

Trifolium appendiculatum, as circumscribed by this paper, sometimes but not always bears this morphological feature. The beak, however, never occurs in *T. variegatum*. The full merits of this trait are not yet well understood. There is no intra-individual variation of the beak. There is no as-of-yet discernable distributional pattern, and there can be both beaked and not-beaked specimens in the same county. Unfortunately, this study has not been able to investigate its heritability nor its function.

Seed size provides compelling support for recognizing these species as distinct, as has been seen in other species complexes in the genus (see, for example, seed size comparisons of *T. barbigerum* Torrey and A. Gray, *T. grayi* Lojacono, and *T. jokerstii* Vincent & R. Morgan [Vincent and Morgan 1998]). Since seeds are not present on all specimens, it was not possible to use this trait in the morphometric analyses. During an attempted common garden experiment utilizing seed from the USDA seed bank, several seeds were found to be noticeably larger than others. After propagation, plants grown from these larger seeds also bore the floral characters of *T. appendiculatum*, including a beaked keel petal. The smaller seeds gave rise to plants with the morphology of *T. variegatum*. The putative *T. appendiculatum* germplasm (USDA PI 593317) has been used in genetic analyses and appears to be genetically differentiated from *T. variegatum* (Ellison, personal communication). A sampling of seed sizes yielded a distinct separation between species ($P < 0.001$, $n=16$, Welch Two Sample t-test, $t=9.9278$), without any overlap of range.

KEY TO *TRIFOLIUM* SPECIES

- 1. Calyx lobes many-toothed, 0–4 (2.6 average) teeth per lobe, teeth 0.8 mm long on average; distal stipules 8–10 mm long; Monterey Co., CA 1. *T. polyodon*
- 1'. Calyx lobes entire, if toothed, 0–3 (0.1 average) teeth per lobe, teeth 0.3 mm long on average; distal stipules 2–10 mm long; western United States, southwestern British Columbia, Canada, and northern Baja California, Mexico 2
- 2. Banner petals 6.3–15.8 mm long, exerted 1.5–8.4 mm beyond tips of calyx lobes; mature seeds 1.9–2.8 mm long; keel petals beaked or not; California and southern Oregon. 2. *T. appendiculatum*
- 2'. Banner petals 3.2–9.7 mm long, included in or exerted 0.1–5.7 mm beyond tips of calyx lobes; mature seeds 1.1–1.6 mm long; keel petals never beaked; British Columbia, Canada, south through Washington, Oregon, Idaho, Montana, California, Nevada, Utah, and Arizona, USA, to Baja California, Mexico 3. *T. variegatum*

TAXONOMIC TREATMENT

- 1. **Trifolium polyodon** Greene, Pittonia 3(17): 215. 1897. *Trifolium tridentatum* var. *polyodon* (Greene) Jeps., Fl. Calif. [Jepson] 2: 292. 1936. — TYPE: USA, CA: Monterey Co., Pacific

Grove, 27 May 1895, *E.L. Greene s.n.* (LECTOTYPE (here designated): NDG 67254!; isolectotypes: NDG 67253!, NDG 67260!) (Fig. 3).

Plants annual, entirely glabrous; roots a taproot, nodulated; stems ascending to decumbent, 10–52 cm in length, 1–2.3 mm in diameter; leaves trifoliolate, dimorphic with gradual transition between proximal and distal leaves; stipules adnate to petiole, free portion lacinate, usually with one tooth longer than the rest; proximal stipules browning with age, each lobe rectangular proximally and triangular distally, 6–14×2–3 mm, margins +/- entire proximally, lacinate distally, apices acute with 1–2 lobes, the longest lobe 2–3.1 mm; distal stipules quarter-circled to ovate, recurved, 4–7×5–6 mm, margins lacinate, with 0–3 apical lobes, the longest lobe 2.5–3.2 mm; petioles 9–55×0.2–0.9 mm; petiolules 0.4–0.7× ≤0.4 mm; proximal leaflets obovate-obcordate, bases cuneate, margins serrate, apices emarginate-mucronate, terminal leaflets 4–10×4–6 mm; distal leaflets elliptic-obovate, bases cuneate, margins serrate, apices rounded, terminal leaflets 13–18.7×6.4–9.2 mm; peduncles 13–54×0.3–0.8 mm; inflorescences axillary, racemose-umbellate, involucrate, 8.2–16×10–17 mm; involucre flat, lacinate, 4.3–6.7 mm radius, lobes indistinguishable from teeth, longest tooth 1.7–2.7 mm; flowers 12–22, in 14 whorls; pedicels 0.5–1× <0.5 mm; calyces 5–7 mm long, tubes 2.3–2.9 mm in circumference, 1.9–2.4 mm long, lobes awl-like to shouldered, tips purple, 3.1–4.6 mm long, apices acute, each bearing 0–4 teeth 0.1–3.1 mm long; petals pink-purple with paler to white tips; banner petals obovate, apices emarginate, 8.3–8.8×2.1–3.2 mm, exerted beyond the tips of calyces 1.5–3.6 mm; wing petals 7.5–8.4 mm long, asymmetrically clawed, claws 4–4.5× ≤0.1 mm, laminae elongate-elliptical, 3.2–4.4×1–1.4 mm, bases shortly auriculate, auricles rounded, petal margins entire, apices rounded; keel petals 6.1–7.1 mm long, asymmetrically clawed, claws 4.4–4.8× ≤0.1 mm; laminae ovate, 1.7–2.4×1–1.3 mm, base rounded, margins entire, apices rounded; stamens diadelphous, connate filaments 6–6.6× ≤0.5 mm; pistils sessile, ovaries 2.3–2.5×0.6–0.7 mm, styles 3.6–4.6× ≤0.1 mm, ovules 2; legumes laterally compressed, 4×2 mm; seeds 2, globular to mitten-shaped, blackish-brown, dark-speckled, 1.9×1.1 mm. 2n=?

Specimens have been collected from two localities (Fig. 1): Pacific Grove (the type locality) and Indian Village, which is approximately three miles away. It has been found in wet grassy locations. All these collections were made at about 120 m in elevation. It flowers in April, May, and June.

In his Ph.D. Thesis, Martin (1943) proposed a new combination, "*Trifolium variegatum* Nutt. var. *polyodon* (Greene) Martin", but this combination was apparently never published.

Representative Specimens. USA. CALIFORNIA: Monterey Co. Pacific Grove, 21 May 1903, *Heller 6759* (ISC); Pacific Grove, 1 May 1909, *Heller 28022*

(WIS); Pacific Grove, 9 May 1909, *Heller 6707* (WIS); horticulturally grown specimens, originally from Indian Village, 20 June 1994, *Yadon s.n.* (MU); Indian Village near Bird Rock, off 17-Mile Drive, 9 April 1998, *Vincent et al. 8195* (MU).

2. ***Trifolium appendiculatum*** Lojac., Nuovo Giorn. Bot. Ital. 15: 181. 1883. —TYPE: USA, California: Coast field, *Lemmon s.n.* (not located at PAL or elsewhere); NEOTYPE (here designated): USA, California: Contra Costa Co., St. Mary's College, 14 May 1933, *J.T. Howell 11227* (neotype: CAS!; isoneotype: WIS!) (Fig. 4).

Trifolium variegatum var. *major* Lojac., Nuovo Giorn. Bot. Ital. 15: 183. 1883. —*Trifolium variegatum* var. *melananthum* f. *major* (Lojac.) McDermott, Ill. Key Amer. Trifolium 76. 1910. —TYPE: USA, California: *Lemmon s.n.* (not located at PAL or elsewhere); NEOTYPE (here designated): USA, California: Tulare Co., hills east of Yokohl Valley, 9 May 1969, *E.C. Twisselmann 15246* (neotype: CAS!; isoneotypes: SBB, RSA).

Trifolium trilobatum Jeps., Bull. Torrey Bot. Club 18: 322. 1891. —*Trifolium variegatum* var. *trilobatum* (Jeps.) Jeps. in McDermott, Ill. Key Amer. Trifolium 73. 1910. —TYPE: USA, California: Sutter Co., base of South Peak, 20 April 1891, *W.L. Jepson 14854* (holotype: JEPS - electronic image!)

Trifolium rostratum Greene, Proc. Acad. Nat. Sci. Philadelphia 47: 547. 1895. —*Trifolium appendiculatum* f. *rostratum* (Greene) McDermott, Ill. Key Amer. Trifolium 92. 1910. —*Trifolium appendiculatum* var. *rostratum* (Greene) Jeps., Man. Fl. Pl. Calif. 539. 1925. —*Trifolium variegatum* var. *rostratum* (Greene) C.L.Hitch. Vasc. Pl. Pacif. N. W. 3: 370. 1961. —TYPE: USA, California: Alameda Co., Oakland, Lake Merritt, 1889, *V.K. Chestnut s.n.* (LECTOTYPE (here designated): NDG!).

Trifolium morleyanum Greene, Erythea 3: 47. 1895. —*Trifolium variegatum* var. *melananthum* f. *morleyanum* (Greene) McDermott, Ill. Key Amer. Trifolium 76. 1910. —TYPE: USA, California: Modoc Co., Morley's Station, 1894, *Baker & Nutting s.n.* (LECTOTYPE (here designated): NDG!).

Trifolium calophyllum Greene, Pittonia 3: 213. 1897. —TYPE: USA, California: Alameda Co., Berkeley, Botanic Garden, 1895, *E.L. Greene s.n.* (lectotype (designated by Gillett 1966): NDG 67170!; isolectotypes NDG 67171!, NDG 67172!, NDG 67214!, NDG 67176!).

Trifolium phaeocephalum Greene, Pittonia 3: 216. 1897. —*Trifolium variegatum* var. *pauciflorum* f. *phaeocephalum* (Greene) McDermott, Ill. Key Amer. Trifolium 78. 1910. —TYPE: USA, California: Butte Co., east of Chico, May 1883, *R.M. Austin s.n.* (lectotype (designated by Gillett 1966): NDG!).

Trifolium splendens A.Heller, Muhlenbergia 1: 115. 1905. —TYPE: USA, California: Monterey Co., Pacific Grove, 7 May 1903, *A. Heller 6691* (holotype: BKL; isotypes: AC - electronic image!,



FIG. 3. Specimen of *T. polyodon*. This horticulturally grown specimen, *Yadon s.n.* (MU), depicts the phenotypic plasticity possible within a population. The calyx lobe teeth are not visible without magnification.

HERBARIUM *dupl. ex:*
LOUISIANA STATE UNIVERSITY
BATON ROUGE

37637



HERBARIUM MU
Trifolium appendiculatum Lojac.
Det.: B.G. Foster & M.A. Vincent 2015

HERBARIUM MU
Trifolium variegatum Nuttall *5.1.*
Det.: Michael A. Vincent 1998

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HERBARIUM
CALIFORNIA STATE COLLEGE AT LOS ANGELES
Trifolium variegatum Nutt. det RMS
Petal tips light purple 7.5P 7/7, base of
flower deep purplish red 7.5RP 3/9.
CALIFORNIA: Tulare Co.: Yokohl Valley road,
17 miles east of Exeter; oak woodland, pastured
425 m elev.
R. M. Straw 2620
Date: 8 May 1965 Collected by: B. Miller, M. Kamin
(82017 67)

FIG. 4. Specimen of *T. appendiculatum*. This specimen, *R.M. Straw, B. Miller, and M. Kamin 2620* (MU), has flowers with beaked keel petals, but these are not visible without magnification. The long banner petals exceeding the calyx lobes, however, are apparent.

BM!, CAS - electronic image! (3 sheets), F - electronic image!, G - electronic image!, ISC!, K, MICH - electronic image!, MSC - electronic image!, NY - electronic image! (two sheets), P - electronic image!, PH (2 sheets)!, US - electronic image!, WIS!)

Plants annual, entirely glabrous; roots a taproot, nodulated; stems ascending, 7–82 cm in length, 0.5–3 mm in diameter; leaves trifoliolate, dimorphic with gradual transition between proximal and distal leaves; stipules adnate to petiole, free portion lacinate, usually with one tooth longer than the rest; proximal stipules browning with age, each lobe rectangular proximally and triangular distally, 7–13×2–5 mm, margins +/- entire proximally, lacinate distally, apices acute with 1–2 lobes, the longest lobe 1.5–3 mm; distal stipules quarter-circled to ovate, recurved, 3.4–10.3×5–6 mm, margins lacinate, with 0–many apical lobes, the longest lobe 0.8–4 mm; petioles 2–73×0.2–1 mm; petiolules 0.3–0.9×≤0.5 mm; proximal leaflets obovate-obcordate, bases cuneate, margins serrate, apices emarginate-mucronate, terminal leaflets 2.9–7×1.5–5 mm; distal leaflets elliptic-obovate, bases cuneate, margins serrate, apices rounded and mucronate, terminal leaflets 5.8–25.9×2.8–10.9 mm; peduncles 17–73×0.3–1.3 mm; inflorescences axillary, racemose-umbellate, involucre, 7.3–21×8–31 mm; involucre flat, lacinate, 3.2–6.5 mm radius, 4–7 lobes, lobes at time indistinguishable from teeth, longest tooth 1.1–3 mm; flowers 4–25, in 1–4 whorls; pedicels 0.3–0.5×<0.5 mm; calyces 3.5–7.4 mm long, tubes 2.2–4.6 mm in circumference, 1.5–3.3 mm long, lobes awl-like to shouldered, tips and shoulders purple, 2.2–5.2 mm long, apices acute, usually toothless, teeth < 1 mm; petals deep purple, white-tipped; banner petals obovate, apices emarginate, 6.3–15.8×1.5–4 mm, exerted beyond the tips of calyces 1.5–8.4 mm; wing petals 5.3–14.2 mm long, asymmetrically clawed, claws 2.9–7×≤0.1 mm, laminae elongate-elliptical, 3–7.3×0.8–1.9 mm, bases shortly auriculate, auricles rounded, petal margins entire, apices rounded; keel petals 4.6–10.6 mm long, asymmetrically clawed, claws 2.8–6.7×≤0.1 mm, laminae ovate, 1.8–3.9×0.8–1.8 mm, base rounded, margins entire, apices rounded to beaked, beaks ≤ 1 mm; stamens diadelphous, connate filaments 3.9–9.8×≤0.5 mm; pistils sessile, ovaries 1.4–4.1×0.3–1.1 mm, styles 2.4–6.2×≤0.3 mm, ovules 2; legumes laterally compressed, 3.6–3.8×2–2.4 mm; seeds 2, globular to mitten-shaped, blackish-brown, black-speckled, 1.9–2.8×1.2–2 mm. 2n=?

Trifolium appendiculatum is found throughout California (Fig. 1), though never east of the Sierra Nevada Mountains, and into southern Oregon (one locality; it is likely rare in Oregon, but our study had poor coverage for this state). It is often collected in open fields, meadows, ditches, marshes, swamps, oak woodlands, and digger pine woodlands, and in rocky, sandy, or loamy soil. Its elevation ranges from 0 to 1400 m. It flowers in March, April, May, and June.

Representative Specimens. **USA. CALIFORNIA:** Butte Co., hills near Big Chico Creek east of Chico, 6 April 1913, *Heller 10710* (CAS); Kern Co., high slope 1 mile east of Poso Creek Narrows, 17 May 1963, *Twisselmann 8251* (CAS); Monterey Co., west of S. Fran., June 1876, *Palmer 71* (GH); San Diego Co., Cuyamaca Rancho State Park. Approx. 0.4 mile NE of intersection of State hwy., 26 June 2005, *Hendrickson 1084* (SD); San Joaquin Co., eastern edge of Wallace, 2 May 1959, *Smith 1063* (CAS); Sutter Co., found growing on damp soil along the edge of West Butte Road, about 1/8 mile north of the Sacramento Outing Club, Sutter Buttes, 19 April 1984, *Ahart 4566* (CAS). **OREGON:** Josephine Co., Grant's Pass, 16 May 1910, *Heller 10030* (CAS), *Heller 10031* (ISC, CAS [2 sheets]).

3. *Trifolium variegatum* Nutt. in Torr. & A. Gray, *Flora of North America* 1: 317. 1838. —TYPE: USA, Oregon: springy places near the mouth of the Wahlamet, *T. Nuttall s.n.* (LECTOTYPE (here designated): NY - electronic image!; isolectotypes: BM!, GH, K) (Fig. 5).

Trifolium spinulosum var. *triste* Torr. & A. Gray, *Fl. N. Amer.* 1: 318. 1838. TYPE: USA, California: Santa Barbara Co., Santa Barbara, *T. Nuttall s.n.* (LECTOTYPE (here designated): BM!).

Trifolium melananthum Hook. & Arn., *Botany of Captain Beechey's Voyage* 331. 1838. —*Trifolium variegatum* var. *melananthum* (Hook. & Arn.) Greene, *Fl. Francisc.* 1: 29. 1891. —*Trifolium tridentatum* var. *melananthum* (Hook. & Arn.) S. Watson, *Proc. Amer. Acad. Arts* 11: 130. 1876. —TYPE: USA, California: *D. Douglas s.n.* (LECTOTYPE (here designated): K 001051289 - electronic image!; isolectotype: BM 010892 - photocopy!).

Trifolium dianthum Greene, *Pittonia* 3: 217. 1897. —TYPE: Canada, British Columbia: Vancouver Island, vicinity of Victoria, 13 May 1893, *J. Macoun 94* (LECTOTYPE (here designated): NDG 67320!; isolectotype: MO!).

Trifolium geminiflorum Greene, *Pittonia* 3: 216. 1897. —*Trifolium variegatum* var. *geminiflorum* (Greene) Vincent, *Madroño* 56: 208. 2009. —TYPE: USA, California: Shasta Co., Lassen's Peak, August 1879, *R. M. Austin s.n.* (LECTOTYPE (here designated): NDG 67301A!; syntype: USA, California: Nevada Co., Donner Lake, July 1890, *C.F. Sonne 3* (NDG 67301B!); syntype: USA, California: Nevada Co., Donner Lake, August 1890, *C.F. Sonne 5* (NDG 67301C!); syntype: USA, California, Amador Co., 1891, *Hansen s.n.* (NDG 67208!).

Trifolium pusillum Greene, *Pittonia* 3: 217. 1897. —TYPE: USA, California: Yosemite Valley, 1881, *C.C. Parry 45* (LECTOTYPE (here designated): NDG!; isolectotype: MO!).

Trifolium subsalinum Greene, *Pittonia* 3: 219. 1897. —TYPE: USA, Nevada: Eureka Co., Palisade, 24 July 1893, *E.L. Greene s.n.* (lectotype (designated by Gillett 1966): NDG!; syntype: USA, Nevada:

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HERBARIUM MU
Trifolium variegatum Nutt.

Det.: B.G. Foster & M.A. Vincent 2015

CALIFORNIA

Trifolium variegatum Nutt.

Mendocino Co., road to Dos Rios from Laytonville.

Stream bed, common but not in dry areas.

J.M. Gillett 12951 June 8, 1966
C.W. Crompton

Chromosome number from
~~this plant~~
this collection $n=8x$

Determined by D. E. Hayley 1966
Annotated by
Department of Agriculture, Ottawa.

DEPARTMENT OF AGRICULTURE, OTTAWA, CANADA

RB 100

FIG. 5. Specimen of *T. variegatum*. This specimen, J.M. Gillett and C.W. Crompton 12951 (MU), depicts a typical example of the species.

Eureka Co., Palisade, 24 August 1896, *E.L. Greene s.n.* (NDG!).

Trifolium ultramontanum Greene, Pittonia 3: 218. 1897. —TYPE: USA, Nevada: Elko Co., Holborn, 16 July 1896, *E.L. Greene s.n.* (lectotype (designated by Gillett 1966): NDG 67201!; isolectotype: NDG 67202!, NDG 67204!); syntype: USA, Nevada: Star Valley, 20 July 1896, *E.L. Greene s.n.* (NDG!).

Plants annual, entirely glabrous; roots a taproot, nodulated; stems prostrate to erect, 2.5–48+ cm in length, 0.1–2.3 mm in diameter; leaves trifoliolate, dimorphic, with gradual or abrupt transition between proximal and distal leaves; stipules adnate to petiole, free portion laciniate, usually with one tooth longer than the rest; proximal stipules browning with age, each lobe rectangular proximally and triangular distally, 2.4–10×0.5–2.1 mm, margins +/- entire proximally, laciniate distally, apices acute with 1–2 lobes, the longest lobe 1–2.5 mm; distal stipules quarter-circled to ovate, recurved or not, 1.7–9.3×0.5–2.5 mm, margins laciniate, with 0–3 apical lobes, the longest lobe 0.6–4.5 mm; petioles 1–95×0.1–1.1 mm; petiolules 0.1–0.8×≤0.5 mm; proximal leaflets obovate-obcordate-cuneate, bases cuneate, margins serrate, apices emarginate-mucronate, terminal leaflets 1.9–5×0.9–2.1 mm; distal leaflets elliptic-obovate, bases cuneate, margins serrate, apices rounded and mucronate, terminal leaflets 2–29×1–11.4 mm; peduncles 4–53×0.1–0.7 mm; inflorescences axillary, racemose-umbellate, involucre, 4.6–12.6×0.8–10 mm; involucre flat, laciniate, 1.5–6 mm radius, 2–7 lobes, at times indistinguishable from teeth, longest tooth 0.6–3 mm; flowers 1–25, in 1–4 whorls; pedicels 0.1–0.5×<0.1 mm; calyces 2.1–6.8 mm long, tubes 1.4–4.1 mm in circumference, 1.9–2.4 mm long, lobes awl-like to shouldered, tips purple or stramineous, 1.5–4.8 mm long, apices acute, occasionally bearing up to 3 teeth per lobe, teeth < 0.6 mm long; petals purple, usually white-tipped; banner petals obovate, apices emarginate, 3.2–9.7×0.6–2.6 mm, included in or exerted beyond the tips of calyces 0.1–5.7 mm; wing petals 3.1–9 mm long, asymmetrically clawed, claws 1.6–5.1×≤0.1 mm, laminae elongate-elliptical, 1.3–4×0.2–1.4 mm, bases shortly auriculate, auricles rounded, petal margins entire, apices rounded; keel petals 3–7.3 mm long, asymmetrically clawed, claws 1.4–4.8×≤0.1 mm, laminae ovate, 12.5×0.4–1.8 mm, base rounded, margins entire, apices rounded-short tipped; stamens diadelphous, connate filaments 2.2–6.6×≤0.5 mm; pistils sessile, ovaries 2–2.9×0.2–1.4 mm, styles 1.1–4.2×≤0.1 mm, ovules 2; legumes laterally compressed, 2.2–4×1.3–2 mm; seeds 2, globular to mitten-shaped, blackish-brown, dark-speckled or not, 1.1–1.6×1–1.2 mm. 2n=16.

Trifolium variegatum is the most widespread of the three species studied, encompassing the ranges of the other two (Fig. 1). It does not appear to have any unique habitat requirements other than some moisture. It is found in grasslands, sandy stream beds, and other open areas, but can also be found in

forests. It occurs in elevations ranging from sea level to 3,000 m. It has been collected in every month from March to October, although this varies depending on the geographic area.

While Gillett (1966) lectotypified a number of Greene's *Trifolium* spp., he did not do so for *T. geminiflorum*. Gillett writes that *T. geminiflorum* Greene was a new name for *T. pauciflorum sensu* Lojac. and then goes on to cite a Lemmon specimen referred to by Lojaccono. However, Lojaccono (1883) was merely commenting on what he thought was a strange specimen (collected by Lemmon) of Nuttall's *T. pauciflorum* and neither intended nor effected a newly published name. Thus, *T. geminiflorum* remained to be lectotypified. It was incorrectly lectotypified by Vincent (2009) because the specimen selected as lectotype (*G. Hansen 1*, Amador Co., April 1892, cited as at NDG but not indicated as having been seen) does not exist at NDG. There is a Hansen specimen (without a number) from 1891 (NDG67208) at NDG, and there are specimens of *Hansen 1* at BM and K (the one at Kew was selected by Vincent as isolectotype). Since, however, *Hansen 1* does not exist at NDG, it is unlikely that Greene saw it, and instead was referring to *Hansen s.n.* (1891, Amador Co.; listed as a syntype above). Thus, Vincent's (2009) incorrect lectotypification is set aside and replaced by the one presented here.

Representative Specimens. **CANADA. BRITISH COLUMBIA:** Vancouver Island, very common especially in wet areas on rocky-grassy bluffs above river, Sproat River Falls north of Alberni, 7 June 1961, *Calder and MacKay 30139* (CAS); Victoria Three miles northeast of Duncan on road to Maple Bay, 19 June 1961, *Calder and MacKay 30732* (CAS). **MEXICO. BAJA CALIFORNIA:** Ensenada: Sierra San Pedro Martir, meadows along road heading south of Vallecitos towards La Encantada, base of Cerro Botella Azul, 19 July 1988, *Boyd et al. 2683* (MU); Laguna Hanson, Constitucion National park, Sierra de Juarez; north end of lake on sandy marsh delta and wet margins of lake, 28 May 1983, *Thorne et al. 55739* (WIS). **USA. ARIZONA:** Graham Co.: Coronado National Forest, Holdout Creek, just above jcn. with Black Rock wash along trail splitting off by corrals, 10 June 1998, *Buegge et al. 338* (ASU). **CALIFORNIA:** San Bernardino Co., Vicinity of Bonanza King Mine, East Slope of Providence Mountains, Mojave Desert, 21 May 1920, *Munz et al. 4020* (MU, CAS); Santa Barbara Co., Santa Cruz Island, Creek below Main Ranch (toward Prisoner's Harbor), 12 October 1958, *Balls 11820* (BM); Inyo Co., Wild Rose Canyon, Panamint Mts., 30 June 1931, *Hoffmann s.n.* (CAS); Tuolumne Co., Tioga Road from Harden Lake to Pareuphene Flat, Yosemite National Park, 13 August 1907, *Eastwood 317* (CAS); San Diego Co., Cleveland National Forest: Northeast of El Cajon Mountain, west of the San Diego River, and south of Barona Mesa, just off of El Cajon Mountain Truck Trail, 7 April 2010, *Rebman et al. 18976* (SD); Kern Co.,

Saddle Springs, north end of the crest of Piute Mountain, 22 June 1962, *Twisselmann 7349* (CAS); Monterey Co., "The Indians" near Jolon, 6 April 1988, *Morgan 1065* (MU). **IDAHO:** Blain Co., Tikura, 22 July 1911, *Nelson and Macbride 1303* (CAS); Kootenai Co., June 1889, *Sandberg s.n.* (MU); Owyhee Co.: S Quicksilver Mountain above Boone Peak, 19 July 1996, *Atwood 21000* (MU, BRY). **MONTANA:** Missoula Co., Missoula, Hay Spur, Geo. F. Fox Ranch, 19 June 1924, *Kirkwood 1756* (MU, ISC, CAS [2 sheets]). **NEVADA:** Nye Co., Along Eden Creek, 2 mi. east on road past Eden Ranch, about 15 mi. due south of Warm Springs, 28 August 1980, *Neese and White 9744* (BRY); Elko Co., Deeth, 23 July 1908, *Heller 9129* (ISC); Washoe Co.: Above the reservoir on Heinz Ranch, northwest slope of Peavine Mountain about 10 miles north of Reno, 21 September 1974, *Howell et al. 50973* (CAS). **OREGON:** Wasco Co., Dalles City, June 8 1897, *Suksdorf 2583* (GH); Lane Co., West of Science building Lane Community College, 9 May 1992, *Love 930* (MU); Clackamas Co., Gladstone, May 1894, *Howell 135* (WIS); Coos Co., 2 mi. west of Charleston opposite Rhodo-Azalea Gds. Nursery, 29 June 1961, *Kimber 122* (WIS). **UTAH:** Box Elder Co., southwest corner of the county, 16 mi. south of Lucine, east of the Pilot Range, Patterson Spring at edge of Great Salt Lake Desert in greasewood belt, 5 July 1976, *Arnow 5013* (BRY, MU); Salt Lake Co., Salt Lake City, July 1880, *Howard s.n.* (BRY). **WASHINGTON:** Ferry Co., Open, springy, gravelly spot 1/4 mi. west of Columbia River, Kettle Falls, 17 June 1939, *Weldert and Boner 151* (CAS [2 sheets]); San Juan Co., Lopez Island, 17 July 1937, *Evans 11524* (MU); Cowlitz Co., Moist bluffs along the Columbia River near Kalama, 7 April 1934, *Thompson 10129* (CAS).

Excluded Taxon

Trifolium variegatum var. *parunuweapensis* S.L. Welsh, Utah Fl., ed. 3. 425. 2003. —TYPE: USA, Utah: Kane Co., ca. 1.5 mi S of The Barracks, subsequent drainage of Parunuweap Canyon, 1450m, 16 July 1992, *S.L. Welsh & K.H. Thorne 25120* (holotype: BRY!).

After comparing the type specimen of *T. variegatum* var. *parunuweapensis* to the type specimen of *T. mucronatum* subsp. *lacerum* (Greene) Gillett (holotype: US - electronic image!, isotype: NY - electronic image!; sometimes synonymized with *T. wormskioldii*), it is evident that this variety is a synonym of *T. mucronatum* subsp. *lacerum*. *Trifolium variegatum* var. *parunuweapensis*, like *T. mucronatum* subsp. *lacerum*, produces rhizomes (visible on paratypes, difficult to distinguish on holotype), has stipules up to 30 mm long, and has three or four ovules. None of these features are ever found in *T. variegatum*, *T. polyodon*, or *T. appendiculatum*.

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